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Refinement of the Background Genetic Map of Xq26-q27 and Gene Localisation for Börjeson-Forssman-Lehmann Syndrome

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A detailed map of genetic markers was constructed around the gene for the X-linked mental retardation syndrome of Börjeson-Forssman-Lehmann (BFLS). A multipoint linkage map of framework markers across Xq26-27, based on CEPH families, was integrated with the physical map, based on a YAC contig, to confirm marker order. The remaining genetic markers, which could not be ordered by linkage, were added to create the comprehensive genetic background map, in the order determined by physical mapping, to determine genetic distances between adjacent markers. This background genetic map is applicable to the refinement of the regional localisation for any disease gene mapping to this region.

The *BFLS* gene was localised using this background map in an extended version of the family described by Turner et al. [1989]. The regional localisation for *BFLS* extends between recombination events at *DXS425* and *DXS105*, an interval of 24.6 cM on the background genetic map. The phenotypic findings commonly seen in the feet of affected males and obligate carrier females may represent a useful clinical indicator of carrier status in potential female carriers in the family. Recombination between *DXS425* and *DXS105* in a female with such characteristic feet suggests that the distal limit of the regional localisation for the *BFLS* gene might reasonably be reduced to *DXS294* for the purpose of selecting candidate genes, reducing the interval for the *BFLS* gene to 15.5 cM.

Positional candidate genes from the interval between *DXS425* and *DXS105* include the *SOX3* gene, mapped between *DXS51*(52A) and *DXS98*(4D-8). *SOX3* may have a role in regulating the development of the nervous system. The HMG-box region of this single exon gene was examined by PCR for a deletion and then sequenced. No deviation from normal was observed, excluding mutations in the conserved HMG-box region as the cause of BFLS in this family.

KEY WORDS: Börjeson-Forssman-Lehmann syndrome, carrier detection, genetic mapping, linkage, prenatal diagnosis, Xq26

INTRODUCTION

Börjeson-Forssman-Lehmann syndrome (BFLS; MIM 301900) is characterised by mental retardation in association with microcephaly, hypogonadism, short stature, obesity, characteristic facial aberrations and, in males, skeletal abnormalities. BFLS is inherited as an X-linked dominant with complete penetrance in males but is partially dominant with variable expressivity in females. Since the first description of this condition by Börjeson et al. [1962], results of linkage mapping have been published for only two families. The affected boys in one family had severe mental deficiency corresponding clinically with the original syndrome description [Ardinger et al., 1984]. The BFLS gene in this family initially mapped distal to *HPRT* in Xq26 and proximal to *F8C* and *DXS15*(DX13) in Xq28 [Mathews et al., 1989a]. The other family had a less severe phenotype with mild/moderate mental retardation and the gene mapped by linkage to the distal portion of

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Xq [Turner et al., 1989] between *DXS10*(6A1) in Xq26 and *DXS105*(cX55.7) in Xq27.

Assuming genetic homogeneity, the *BFLS* gene was placed at Xq26-27 by the combined analysis of these two families: distal to *HPRT* and proximal to *DXS105*. Pooled data found no recombinants with the probe *DXS51*(52A) and a combined peak lod score of 3.98 at $\theta = 0.00$ [Mathews et al., 1989b]. However, subsequent analyses have shown that the recombinant defining the proximal limit at *HPRT* [Mathews et al., 1989a] was questionable [Mathews et al., 1993] and the recombinant defining the proximal limit at *DXS10* (near *HPRT*) in the present family [Turner et al., 1989] was incorrect [Davies et al., 1990]. The proximal limit to the BFLS gene therefore remains undefined. Although there is no evidence for genetic heterogeneity since both severe and mild forms of BFLS map to the same region, the gene has been mapped in only two published families.

The present study applied microsatellite markers to an extended version of the family first described by Turner et al. [1989] to determine a proximal limit to the BFLS gene localisation. This was based on a comprehensive map of markers surrounding and within the BFLS interval established by linkage and physical mapping.

MATERIALS AND METHODS

The Family

Descriptions of the affected males have been published previously [Turner et al., 1989]. Individual identifiers on the pedigree have been preserved to correspond with earlier clinical descriptions (Fig. 1). In the original description II-8 was thought a likely new mutation, having 3 normal brothers II-1, 2, 3. The finding that III-13 had Klinefelter syndrome explained his mild learning difficulties. A number of Klinefelter manifestations overlap those of BFLS expressed in a male with two X chromosomes. These include mild mental retardation, gynaecomastia and small testes. II-6 cannot be inferred with certainty as a carrier of the *BFLS* gene, due to the ambiguity of her son's phenotype. III-12 was considering having a family and raised the question of her carrier status. It was decided that the question might be resolved by extending the linkage analysis to II-1, 2 and 3 to determine if they had received a different region of the X chromosome around the gene for BFLS than the obligate carrier sister II-8.

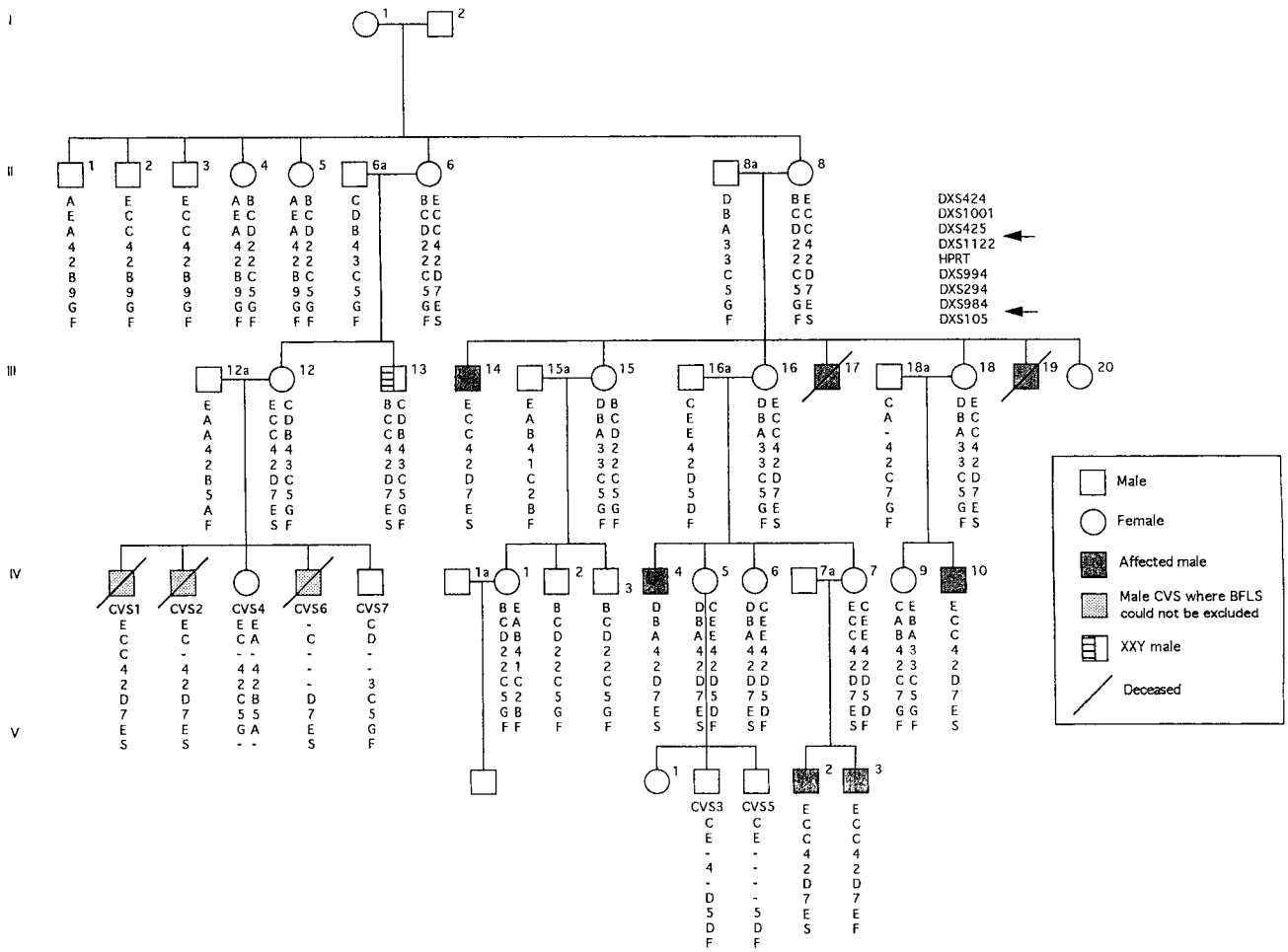

Fig. 1. Extended pedigree of the kindred.

The feet of affected males in this family are short with clawed or flexed toes and a broad forefoot. This abnormality and subsequent discussion within the family led to the statement from II-8 that her feet were similar to those of her sisters II-4 and II-6. The feet of II-8, an obligate heterozygote, and two affected males, III-14 and IV-4 have been depicted previously [Turner et al., 1989]. Short toes were also found in the females II-6 and IV-5, and the obligate carrier III-18. This phenotypic manifestation appears to be variably expressed in female carriers of *BFLS* and when present may be suggestive of carrier status in potential female carriers. III-13 has hammer toes originating high and hanging over the ball of the foot. His mother (II-6), sister (III-12), and niece (CVS4—at 2 years of age) all have the same anomaly. The feet of IV-5 and her daughter V-1 also demonstrate the typical short, flexed toes seen in affected males. However, this anomaly alone cannot be considered conclusive for prediction of carrier status.

Studies on the original family are now extended to include II-1 to II-6, III-12, III-13, and IV-1 to IV-3. Carrier studies were made possible for individuals II-4, II-5, III-12, III-13, III-15, IV-1, IV-5, IV-6, IV-9, and V-1 and prenatal diagnosis on chorionic villus samples (CVS) from 5 pregnancies in generation IV—CVS1, CVS2, CVS4, CVS6, and CVS7; and two pregnancies in generation V—CVS3 and CVS5. The CVS samples were numbered in chronological order.

DNA Analysis

Additional family members were genotyped at RFLP loci (*DXS86, DXS10, DXS51* and *DXS105*) as described by Turner et al. [1989]. PCR based microsatellite markers were genotyped as described previously [Gedeon et al., 1991]. Primer sequences were as given for *DXS102, DXS294,* and *DXS300* [Gedeon et al., 1991, 1992]; for *DXS984, DXS994,* and *DXS1001* [Weissenbach et al., 1992]; *DXS292* and *DXS297* [Richards et al., 1991]; *DXS424* and *DXS425* [Luty et al., 1990]; *HPRT* [Edwards et al., 1991]; *DXS1122* [Donnelly et al., 1994]; and *DXS691* and *DXS692* [Lasser et al., 1993]. Fetal sexing by PCR was carried out using Y specific primers as described by Kogan et al. [1987] and confirmed by karyotyping.

Linkage Analysis

The dinucleotide repeat markers *DXS102, DXS294,* and *DXS300* were characterised as described by Gedeon et al. [1991, 1992] from (AC)n positive lambda phage or plasmid, targetted because they mapped within the region of interest on the cytogenetic based physical map [Suthers et al., 1990]. The dinucleotide repeat marker *DXS1122* was characterised from a randomly selected (AC)n positive clone which mapped by chance to a region close to and proximal to the *BFLS* regional localisation on the multipoint linkage map [Donnelly et al., 1994]. *DXS102, DXS294, DXS300,* and *DXS1122* were genotyped on the 40 CEPH (Centre d'Etude du Polymorphisms Humain) families as part of the present study. Available genotypes for the remaining markers were extracted from the CEPH database, and a multipoint background map of framework markers was constructed as described by Kozman et al. [1993]. This marker order was compared for concordance with that determined from the physical map. The order of the remaining markers was based on the physical map and using this order a comprehensive genetic map was constructed to determine genetic distances between markers.

Several markers included in this background map were genotyped on the extended BFLS family. Two-point lod scores involving the BFLS locus were determined by MLINK analysis [Lathrop and Lalouel, 1984]. A mutation rate of 10^{-4} was used since it was uncertain whether II-8 was a new mutation.

Physical Mapping

The markers *DXS102, DXS294,* and *DXS300* were positioned by PCR analysis onto the YAC contig map of Little et al. [1992]. The physical order of the remaining markers from the genetic map for the Xq26-q27 region was subsequently established from the extended YAC contig [Pilia et al., 1996]. This confirmed the order for markers in the framework genetic map (ordered by linkage with odds of 1,000:1 or more) and established the order for the remaining markers included within the comprehensive genetic linkage map.

Candidate Gene Analysis

The *SOX3* gene, related to *SRY,* is expressed at high levels in neuronal tissues during development in the mouse and is found in human fetal brain and spinal cord [Stevanovic et al., 1993]. The gene has been mapped to Xq26-q27, between *DXS51* and *DXS98,* on somatic cell hybrids and has been suggested as a candidate gene for BFLS [Stevanovic et al., 1993]. The amino acid sequence of the HMG-box region is identical to the mouse *Sox-3* protein and may be the most closely related to SRY genes. All mutations described in the SRY gene have thus far been located in the HMG box-like motif, suggesting loss of DNA binding capacity of the mutated protein [Poulat et al., 1994]. The HMG portion of the SOX3 single exon gene can be amplified in genomic DNA by PCR using published primers S3F1 and S3F3 [Stevanovic et al., 1993]. The resultant products of approximately 300 bp were screened for mutation on an agarose gel to detect any gross changes such as a deletion, screened by SSCA on polyacrylamide gels to detect conformational changes arising from point mutations, and sequenced to detect point mutations which may have been missed by SSCA. Comparison was made between an affected male and an unaffected male.

RESULTS

The Background Map

The background genetic map is given in Table I. The genetic distances between markers (in centi-morgans) established by linkage were cen-*DXS424,DXS1212,DXS1001*-(8.5)-*DXS425*-(6.1)-*DXS994*-(0.0)-*DXS42*-(0.1)-*DXS100,DXS1047,DXS1122*-(4.4)-*DXS79,HPRT*-(1.1)-*DXS86*-(0.8)-*DXS144,DXS300, DXS1062,DXS1206*-(3.0)-*DXS294*-(1.3)-*DXS51*-(0.6)-

TABLE I. Comprehensive Genetic Linkage Map of the Xq25-q27 Region

Marker	cM	Odds against inversion
DXS1212	0.0	1:1
DXS1001	0.0	1:1
DXS424	8.5	10^{16}:1
DXS425	6.1	10^{6}:1
DXS994	0.0	1:10
DXS42	0.1	1:1
DXS100	0.0	1:1
DXS1047	0.0	1:1
DXS1122	4.4	1.2:1
DXS79	0.0	1:1
HPRT	1.1	10^{3}:1
DXS86	0.8	10:1
DXS144	0.0	1:1.3
DXS300	0.0	1:1
DXS1062	0.0	1:1
DXS1206	3.0	1.2:1
DXS294	1.3	1.3:1
DXS51	0.6	1:1
DXS1211	0.5	1:1
DXS1192	0.0	1:1
DXS102	1.7	10^{4}:1
F9	0.0	1:1
DXS1232	1.3	1:1
DXS984	3.7	10^{3}:1
DXS105	0.0	1:1
DXS1227	7.1	1.3:1
DXS98	6.0	10^{2}:1
DXS292	2.8	10^{2}:1
DXS369	1.6	1.3:1
DXS1200	0.0	1:1
DXS297	0.0	1:1
DXS998		

DXS1211-(0.5)-*DXS1192,DXS102*-(1.7)-*F9,DXS1232*-(1.3)-*DXS984*-(3.7)-*DXS105,DXS1227*-(7.1)-*DXS98*-(6.0)-*DXS292*-(2.8)-*DXS369*-(1.6)-*DXS1200,DXS297,DXS998*-qter. The genetic map included 22 PCR based markers and extended from DXS424 to DXS297, a distance of 50.6 cM. The linear order of markers was established from the framework linkage map and/or placement of markers onto the YAC contig.

Application of markers from this map to other BFLS families can be used to further reduce the regional localisation for the BFLS gene on both the physical and genetic maps. Locus order and distances between markers on these maps form the basis for current diagnostic application using the technique of flanking marker analysis. However, caution is urged. Few BFLS families have been mapped and prior to diagnosis by linkage the putative BFLS gene should be mapped in each new family to ensure that the gene does in fact map to the Xq26-q27 region.

Two-Point Linkage Analysis

The kindred is shown in Figure 1. Two-point lod scores between *BFLS* and markers spanning the regional localization are given in Table II. The maximum two-point lod score was 2.61 with *DXS294* at a recombination fraction of zero. Recombination events were detected at *DXS425* in individual IV-4 and at *DXS105* in individual V-3. These data are consistent with the placement of the *BFLS* gene in this family between *DXS425* and *DXS105*.

Haplotype analysis of the pedigree demonstrates that CVS4 carries a recombinant maternal chromosome within the *BFLS* region. The crossover event occurred between *HPRT* and *DXS294* so that the proximal markers were the grandmaternal alleles and the same as those found in the affected males in the remainder of the pedigree. The distal set of markers including *DXS994*, were grandpaternal alleles. This recombination event places *DXS994* distal to the recombination point between *HPRT* and *DXS294* but does not provide order in relation to *DXS294*.

Diagnosis in this family was carried out using markers known to flank the disease gene. Markers from within the regional localisation for BFLS cannot be used for diagnosis alone because their recombination frequency with BFLS is unknown, and for many of

TABLE II. Two-Point Lod Scores Between BFLS and Marker Loci in the Xq24-q27 Region

Marker loci	Recombination fraction								
	0.0	0.01	0.05	0.1	0.2	0.3	0.4	Zmax	θmax
DXS424	−1.69	0.28	0.83	0.95	0.86	0.60	0.29	0.95	0.12
DXS1001	−2.60	−0.61	0.02	0.23	0.34	0.31	0.19	0.34	0.22
DXS425	−1.69	0.28	0.83	0.95	0.86	0.60	0.29	0.95	0.12
DXS1122	1.75	1.72	1.60	1.43	1.08	0.69	0.31	1.75	0.00
HPRT	0.83	0.81	0.76	0.69	0.55	0.39	0.21	0.83	0.00
DXS86	1.58	1.55	1.44	1.30	1.01	0.70	0.37	1.58	0.00
DXS10	0.98	0.96	0.88	0.79	0.60	0.41	0.22	0.98	0.00
DXS994	2.01	1.97	1.81	1.61	1.20	0.77	0.34	2.01	0.00
DXS294	2.61	2.56	2.37	2.12	1.61	1.06	0.50	2.61	0.00
DXS51	2.35	2.31	2.15	1.94	1.49	1.0	0.48	2.35	0.00
DXS102	0.83	0.81	0.76	0.69	0.55	0.39	0.21	0.83	0.00
F9	1.45	1.43	1.32	1.18	0.87	0.55	0.23	1.45	0.00
DXS984	2.61	2.56	2.37	2.12	1.61	1.06	0.50	2.61	0.00
DXS105	−1.39	0.57	1.09	1.17	1.00	0.69	0.33	1.17	0.10
DXS98	−1.67	0.30	0.85	0.97	0.87	0.61	0.29	0.97	0.11
DXS297	−0.03	−0.03	−0.03	−0.02	−0.02	−0.01	−0.00	0.00	0.50

these markers, too great for accurate diagnosis. Genotyping a selection of markers from within the regional localisation does, however, guard against the chance of undetected double crossover between the flanking markers which could otherwise give a misleading diagnostic result using flanking markers. The females IV-5 and IV-6 were predicted to be definite carriers because they carried the same haplotype as their brother IV-4, which in this family is responsible for the disease. In contrast, II-4, II-5, and III-15 and all of her descendants did not carry the haplotype and can be excluded as potential carriers. Although II-6, III-12, and III-13 carry the same haplotype as all of the affected males and obligate carrier females, their carrier status remains uncertain. The possibility remains that II-8 may be a new mutation, a question which cannot be resolved since none of her brothers II-1, II-2, or II-3 carry the haplotype associated with the disease. The phenotype of III-13, who carries this haplotype, could have resolved this issue had he not had Klinefelter syndrome, since the additional X may modify the BFLS phenotype to that seen in female carriers but with ambiguity due to superimposed features of Klinefelter syndrome.

The third fetus of III-12 (CVS4) was female and provided potentially valuable information on the *BFLS* localisation. Since crossover between flanking markers had occurred, carrier status could not be predicted. This pregnancy was not terminated because female carriers do not manifest the degree of mental retardation seen in affected males. The feet of II-6 and III-12 and her liveborn daughter (CVS4) are phenotypically similar to those of affected males and other obligate female carriers in the family. A recombination event between *HPRT* and *DXS294* in the maternally derived haplotype of CVS4, who by clinical observation may be a carrier, suggested that the *BFLS* gene may lie between *DXS425* and *DXS294,* in an interval 15.5 cM in length. This location of BFLS would exclude involvement of the *SOX3* gene which maps distal to *DXS51.*

Candidate Gene Analysis

No deletion or point mutation was detected in the BFLS patient screened and sequenced for the HMG domain of the single exon *SOX3* gene. The exclusion of involvement of the HMG domain of this gene in the BFLS patient examined suggests that this region of *SOX3* may be discounted as a candidate for *BFLS.* A lymphoblastoid cell line LL556 derived from a male with haemophilia and mental retardation is deleted between *F9* and *DXS369* [Rousseau et al., 1991]. The *SOX3* gene mapping between *DXS51* and *DXS98* partly overlaps this deletion between *F9* and *DXS98* and may be involved in the phenotype of this male. Given the recombination event observed in CVS4 together with the possibility of this female carrying the *BFLS* gene based on the phenotype of the feet, the *BFLS* gene may in fact lie proximal to *DXS294* and be physically excluded from overlapping with the *SOX3* gene. However, examination of the HMG domain alone cannot rule out *SOX3* as a candidate gene for BFLS but exclusion by position must await detection of an unequivocal recombination event in a male from a family with BFLS.

DISCUSSION

The closest proximal marker flanking the BFLS gene has now been established in this family as *DXS425.* This marker represents the current proximal boundary for the regional localisation of the *BFLS* gene for use in diagnosis. The maximum two-point lod score is now 2.61 at zero recombination with the marker *DXS294,* an increase from 2.1 at zero recombination with *DXS51* as previously reported for this family [Turner et al., 1989]. Prenatal diagnoses performed in seven pregnancies in this family relied on the genetic background map compiled from a combination of CEPH genotype data and physical mapping on a YAC contig. Flanking markers *DXS425* and *DXS105* define the 24.6 cM BFLS gene interval associated with the mental retardation and clinical findings in affected males of the family.

Most affected patients have severe mental retardation [Mathews et al., 1989a]. In the family of Turner et al. [1989] the phenotype is less severe, with mild/moderate mental retardation and without short stature and microcephaly. Despite this variation in expression there is no evidence for locus heterogeneity since the severe and mild forms map to the same Xq26-27 region. Variable expression between families can be accounted for by allelic heterogeneity.

Huang et al. [1991] and Malmgren et al. [1993] have mapped genes for other mental retardation syndromes to this same region. The family studied by Huang et al. [1991] has been described in detail by Pettigrew et al. [1991]. The family of Malmgren et al. [1993] was described in detail by Gustavson et al. [1993]. Malmgren et al. [1993] made comparisons between these disorders and speculated that there existed the possibility of a contiguous gene syndrome in the area. If we accept that BFLS is an entity distinct from the disorders described by Gustavson et al. [1993] and Pettigrew et al. [1991], then a contiguous gene syndrome to account for all of the above would require the presence of a series of overlapping submicroscopic deletions or some other genetic mechanism which affects more than one contiguous locus.

The availability of a YAC contig which has been integrated with the genetic map provides the basis for searching for expressed sequences by exon trapping or direct selection. Alternatively, partial cDNA sequences (ESTs) which map into the region of interest on somatic cell hybrid panels and confirmed by placement onto the YAC contig can be used to probe cDNA libraries in order to recover coding sequences. Integration of the contig with the genetic map provides a defined set of overlapping clones, one or more of which will carry the BFLS gene. The boundary for the regional localisation will contract as new markers distal to *DXS425* and proximal to *DXS105* are characterised. This will allow the precise identification of recombination points in existing families and the analysis of additional BFLS families might uncover crossover points closer to the *BFLS* gene than those presently known. This will minimise the regional localisation of *BFLS* prior to testing candidate expressed sequences for point mutations in affected families. So far, no submicroscopic deletions associated with BFLS have been detected. These would

expedite the search for the BFLS gene, since they would reduce the regional localisation more effectively than linkage studies. If a proportion of BFLS mutations do exist as submicroscopic deletions they should be detectable as the marker density within the delineated region increases and these markers are applied to BFLS families.

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